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Interferon vs. Adenine Arabinoside 5′-Monophosphate in Patients With Anti-HBe-Positive Chronic Hepatitis

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Anti-HBe-positive patients with precore mutants may have severe, progressive liver disease. Therapy with interferon has been effective, but relapses are frequent. To evaluate and compare two antiviral treatments, lymphoblastoid interferon (ly-IFN) and adenine arabinoside 5′-monophosphate (ARA-AMP), 20 patients with anti-HBe-positive chronic hepatitis (5 cirrhosis and 15 CAH) and viral replication (HBcAg in the liver and HBV DNA in serum) were treated. Patients were randomized into two groups: 11 patients received ARA-AMP, 5 mg/kg/day during 7 weeks, and 9 received human ly-IFN, 5,000,000 units, three times per week, during 4 months. Baseline clinical, biochemical and histological features were not significantly different between the two groups. At the end of therapy, 8 (89%) patients in the interferon group and 5 (45%) in the ARA-AMP group showed normal ALT levels and no HBV DNA in serum by a liquid hybridization assay ($P < 0.05$). At 1 year of follow-up, a persistent response was observed in 33% of ly-IFN patients and in 27% of ARA-AMP patients, a transient response in 56% and 18%, and nonresponse in 11% and 55%, respectively. HBV DNA remained detectable by polymerase chain reaction (PCR) in 19 of the 20 patients. Among the responders, an improvement in histological lesion and the disappearance of intrahepatic HBcAg were observed; in the nonresponders, histological lesion remained stable or worsened. In conclusion, the efficacy of interferon and ARA-AMP was similar in treating anti-HBe-positive chronic hepatitis. Although interferon treatment led to initial improvement in a larger number of patients, there was a much higher rate of relapses with this drug.

KEY WORDS: interferon, adenine arabinoside 5′-monophosphate, anti-HBe

INTRODUCTION

Molecular biological studies have led to the discovery of one or more mutations in the precore region of the hepatitis B virus (HBV) genome that inhibit the production of HBeAg [Brunetto et al., 1989]. Most anti-HBe-positive patients have inactive liver disease and no evidence of HBV replication and will not benefit from antiviral therapy. However, a percentage of chronic hepatitis B patients with mutations in the precore region of HBV that prevent the secretion of HBeAg may continue to have active liver disease and progress toward more severe forms of liver injury [Hadziyannis et al., 1983]. Patients with the stop codon mutation are also more likely to have elevated alanine aminotransferase (ALT) levels and HBV DNA in serum. The predominant mutation in these patients is a G to A change at nucleotide 1986, creating a stop codon in codon 28 [Carman et al., 1989]. In Mediterranean countries, such as Greece, Italy and Spain, approximately 60–80% of all treated patients with chronic hepatitis B fall into this category [Brunetto et al., 1989; Alberti et al., 1991]. Conflicting data have accumulated on the response to treatment in these patients. Interferon therapy can suppress HBV replication and induce remission of liver disease, but the incidence of relapses is high.

Adenine arabinoside 5′-monophosphate (ARA-AMP) appears to be an alternative treatment for HBV infection in HBeAg-positive patients, especially in Europe [Marcellin et al., 1995]. To date there is no experience with this drug in anti-HBe-positive patients with viral replication. The present study compares the effectiveness of two antiviral therapies, ARA-AMP and human lymphoblastoid interferon (ly-IFN), with respect to inhibition of HBV replication in anti-HBe-, HBV DNA-positive chronic hepatitis B cases.

PATIENTS AND METHODS

Patients

Twenty patients were enrolled in the study. All had been positive for HBsAg and anti-HBe for at least 6 months and had HBV DNA in serum by dot hybridization and HBcAg in the liver. The inclusion criteria also required elevation of serum ALT levels to at least 1.5 times the upper normal limit; histological features of

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chronic hepatitis or cirrhosis; and negative tests for anti-HDV, anti-HIV, and anti-HCV. In addition, patients had well-compensated liver disease.

Methods

HBsAg, HBeAg, anti-HBe, anti-HCV, anti-HDV, and anti-HIV were detected by ELISA using commercial kits (Abbott Laboratories, North Chicago, IL). Serum hepatitis B virus DNA (HBV DNA) was determined using a dot blot hybridization procedure [Rodriguez-Frias et al., 1994] and was measured quantitatively by a liquid hybridization assay (Genostics; Abbott Laboratories). HBV DNA was also evaluated by polymerase chain reaction (PCR) as described previously [Rodriguez-Frias et al., 1994]. Mutations of the precore region were studied by direct sequencing of the PCR products using a fluorescent PCR method. Staining analysis for HBcAg in the liver was performed with a peroxidase-antiperoxidase technique in formalin-fixed, paraffin-embedded sections from liver biopsy (Dako Corporation, Santa Barbara, CA).

Study Design and Treatment Regimen

After a 6 month screening period, patients were randomized to receive human ly-INF (Wellferon; Wellcome Foundation Limited, Beckenham, U.K.) at 5,000,000 units, given subcutaneously, 3 times per week for 4 months, or ARA-AMP (Vidarabine, Parke Davis, Courbevoie, France) at 5 mg/kg/day, by the intramuscular route, at 12 hour intervals for 7 weeks. The trial was approved by the ethics committee of the institution where it was conducted, and informed consent was obtained.

The baseline characteristics of the two groups are shown in Table I. Patients were followed every 2 weeks during therapy and, afterwards, every 2 months for 1 year. Clinical evaluation, complete blood count, serum ALT levels, and HBV serology (HBsAg, HBeAg, anti-HBe, and HBV DNA) were carried out on each visit. HBV precore mutants were studied in serum samples before and after therapy. Liver biopsies were obtained before therapy and at the end of follow-up. Histological assessment was made under code by a single pathologist blinded with respect to treatment group and to the chronological order of biopsies. Histological activity was measured by the Knodell index. HBcAg analysis in the liver was carried out by an immunoperoxidase technique.

The patients were classified into three groups according to an evaluation made at the completion of the 12 month follow-up period: Patients were considered to be responders if there was continuous ALT normalization and HBV DNA clearance as detected by liquid dot hybridization during the therapy period and at 12 months of follow-up; patients were categorized as relapsers when transient ALT normalization and HBV DNA clearance during therapy were followed by reactivation; and patients categorized as nonresponders when ALT remained elevated and HBV DNA remained detectable.

Statistical Analysis

Fisher's exact test and Student's t test were used for statistical comparisons. A P value of less than 0.05 was considered significant.

RESULTS

Patients were distributed evenly among the two groups in terms of number, age, sex ratio, male homosexuality, mean ALT level, HBV DNA at entry, and baseline liver histology (Table I). All had baseline ALT levels equal to or greater than 1.5 times the upper normal limit and HBV DNA levels over 5 pg/ml.

Three principal mutations were found in the precore region: M1 (substitution G→A at position 1896, codon 28, change Trp for a stop codon), M2 (substitution G→A at position 1899, codon 29, change Gly for Asp), and M3 (substitution C→T at position 1817, codon 2, change Gln for a stop codon). The distribution of these variants is shown in Table 1. None of the patients was withdrawn from the study during treatment or follow-up.

Response to Therapy

Eight of nine (89%) patients in the ly-IFN group and 5 of 11 (45%) in the ARA-AMP group showed normalized ALT levels and cleared HBV DNA at the end of therapy ($P < 0.05$). In contrast, no difference was seen with respect to serum HBsAg, which remained detectable in all cases.

At 12 months of follow-up, complete response was observed in 3 (27%) patients treated with ARA-AMP and in 3 (33%) treated with ly-IFN. A transient response followed by relapse was seen in 2 (18%) ARA-AMP-treated cases and in 5 (56%) ly-IFN-treated cases. There was no response in 6 (55%) ARA-AMP-treated patients and in 1 (11%) ly-IFN-treated patient. Only one patient (receiving ARA-AMP) tested negative for HBV DNA by PCR. The final evaluation of efficacy is described in Table II. No patient had an ALT flare-up to more than twice the baseline value during therapy.

Response to ly-IFN or ARA-AMP was not influenced by any of the variables that we analyzed: age, sex, baseline ALT level, liver histology, HBV DNA level, or type of HBV variation (Table III). At the end of follow-up, patients presented the same main (principal prevalent) mutations of the HBV precore region as were initially demonstrated.

Liver Histology

After follow-up, liver biopsy specimens were obtained from 18 patients (9 treated with ly-IFN and 9 with ARA-AMP), and the paired specimens were evaluated to assess changes in histological activity. Among the 18 patients, 11 had a persistent or transient response. All these responders showed a reduction in portal and periportal inflammation, lobular injury and piecemeal necrosis, but only one of these, piecemal necrosis, showed a significant statistical improvement ($P < 0.05$; Table IV). No significant changes in pre- and post-treatment biopsies were found among the nonresponders. HBcAg by immunoper-

TABLE I. Clinical and Laboratory Data for the Patients

	ARA-AMP (n = 11)	Interferon (n = 9)
Sex (% male)	91	67
Age (years; mean ± SD)	48.45 ± 12.20	41.33 ± 7.50
ALT (IU/liter; mean ± SD)	137.18 ± 51.04	143.00 ± 63.15
Male homosexuality	0	0
HBV DNA (pg/ml; mean ± SD)	49 ± 118.30	23 ± 45.07
Liver histology		
CAH	7	8
Cirrhosis	4	1
HBcAg + hepatocytes	11	9
HBV variants		
M1	5	2
M1 + M2	3	5
M2 + M3	1	—
Not determined	2	2

TABLE II. Final Evaluation of the Efficacy of Therapy

	ARA-AMP (n = 11)	Interferon (n = 9)
Complete response (%)	3 (27)	3 (33)
Relapse (%)	2 (18)	5 (56)
Nonresponse (%)	6 (55)*	1 (11)*

*$P < 0.05$.

oxidase was found in 2 of the 11 (18%) responders and in 6 of the 7 (85%) nonresponders.

Side Effects

Interferon and ARA-AMP were generally well-tolerated; it was not necessary to suspend treatment in any patient because of side effects. Flu-like symptoms occurred in 5 of the 9 (55.5%) interferon-treated patients during the first 2 weeks of therapy. Mild side effects, such as myalgia, were seen in 3 (27%) patients receiving ARA-AMP. No reduction in the initial doses of interferon or ARA-AMP was required.

DISCUSSION

The results from this study show that treatment with interferon or ARA-AMP at the dosing regimens used modifies the clearance of HBV DNA in anti-HBe-positive patients. The two drugs manifested similar efficacy in achieving a complete response. Results for interferon similar to these were obtained in four different trials [Alberti et al., 1991; Hadziyannis et al., 1990; Fattovich et al., 1992; Pastore et al., 1992]. During treatment, the percentage of patients with normalized ALT and clearance of HBV DNA varied, ranging between 53% and 68%. However, the beneficial effect of interferon was transient in the majority of cases, and relapses were frequent, ranging from 27% to 89%. The low rate of relapse in one of these studies might possibly be explained by the fact that few of the patients included had cirrhosis [Fattovich et al., 1992]. Interferon-induced recoveries are different in HBeAg-positive patients and in anti-HBe-positive patients. Twenty-three percent of patients with prevalent mutants and 47% of patients with prevalent wild-type HBVs responded to interferon. Relapses occurred in 86% and 19% of treated patients with a prevalent precore mutant and a prevalent wild-type HBV, respectively. High levels of precore mutant HBV (>20% of total viremia) were associated with a transient response to interferon, suggesting that the HBV precore mutant escapes immunomodulation more efficiently than the wild-type virus [Brunetto et al.,

TABLE III. Clinical and Laboratory Features in Relation to Response to Therapy

	Responders (n = 13)	Nonresponders (n = 7)	*P*
Sex (% male)	69	100	ns
Age (years)	46.34	44.12	ns
ALT (IU/liter; mean ± SD)	143 ± 34	136 ± 82	ns
HBV DNA (pg/ml)	17.00 ± 37.87	76.50 ± 145.21	ns
Liver histology			
CAH	10	5	ns
Cirrhosis	3	2	ns
HBcAg + hepatocytes	13	7	ns
HBV variants			
M1	3	4	ns
M1 + M2	8	—	ns
M2 + M3	—	1	ns
Not determined	2	2	ns

TABLE IV. Liver Biopsy Changes in 11 Responders

	Before therapy (n = 11)	After therapy (n = 11)	P
Mean Knodell index	9.39 ± 3.37	7.70 ± 4.13	ns
Portal inflammation	2.60 ± 1.57	2.10 ± 1.52	ns
Lobular injury	2.00 ± 1.05	1.80 ± 1.03	ns
Piecemeal necrosis	2.80 ± 0.63	2.20 ± 1.30	<0.05
Fibrosis	2.00 ± 1.33	2.20 ± 1.31	ns

1993]. Therefore, chronic hepatitis B should be treated as early as possible in its natural course, before the HBV precore mutant becomes selected as the prevalent virus [Brunetto et al., 1993; Bonino and Brunetto, 1993; Carman et al., 1993; Bertoletti et al., 1994].

Experience with ARA-AMP therapy is still quite limited. In a study by Marcellin et al. [1995], a complete and sustained response to ARA-AMP administration in chronic hepatitis B was seen only in a group of patients with low HBV replication (HBV DNA <100 pg/ml). In anti-HBe-positive patients, even with low levels of HBV DNA, sustained response was infrequent, in contrast to the case in HBeAg-positive patients. However, in anti-HBe-positive patients, the response at the end of therapy was higher in patients with low pretreatment HBV DNA levels and was similar to that in HBeAg-positive cases [Marcellin et al., 1995]. Although our study showed a lower rate of complete response with ARA-AMP treatment than that usually seen with ly-IFN, ARA-AMP should be considered for treating chronic hepatitis B patients who do not respond to, who have contraindications to, or who develop side effects to ly-IFN. In particular, ARA-AMP might be useful in treating liver or kidney transplant recipients, in whom ly-IFN might increase the risk of rejection.

In conclusion, doses and schedules of ly-IFN that induce a sustained response in HBeAg-positive patients show similar effectiveness in anti-HBe-positive chronic hepatitis B patients. Therefore, future treatments should include more efficacious drugs and should be begun as early as possible during the natural course of the disease.

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